Art-Related Remarks

35 U.S.C. § 103

Applicant respectfully traverses the rejection of claims 1-20 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Karkmann *et al.*, *J. Immunol. Meth.*. 230: 113-20 (1999) in view of Roth *et al.*, U.S. Patent No. 5,902,272; and Lollini *et al.*, *Immunology Blackboard* 1 (1998) in view of Roth *et al.*

The present claims recite the use of a medium comprising a chaotropic agent to wash cells labeled by tyramide deposition methods. As discussed by Applicant in the telephonic interview, it is acknowledged by the Examiner that the primary references relied upon in the rejection do not disclose the use of chaotropic agents in tyramide staining methods. Moreover, the secondary Roth *et al.* patent, which is cited by the Examiner for allegedly disclosing the use of chaotropic agents in tyramide staining methods, is quite careful to avoid contacting cells with any chaotropic agent.

As noted in column 4, lines 35-39, the methods disclosed in the Roth *et al.* patent are designed for staining of cells immobilized to a surface, and not for the labeling of cells in solution for flow cytometry. Chaotropic agents are used in "quantitation aspects" discussed, e.g., beginning in column 7, line 46. In these aspects, a sample of the solution phase is removed from the immobilized cells, and it is only to this removed solution phase that a chaotropic agent is added. Thus, the cited publications, alone or in combination, fail to disclose or suggest methods in which cells are contacted with a wash solution comprising a chaotropic agent, as required by the instant claims. As Applicant discussed in the telephonic interview, this wash step can advantageously reduce background staining in the claimed tyramide amplification labeling methods.

During the telephonic interview, the Examiner requested that the claims explicitly indicate that the cells are contacted with a solution comprising a chaotropic agent. Applicant has amended the claims accordingly. Applicant respectfully submits that this contacting was inherently part of the "wash" step present in the claims as originally drafted; hence, this

amendment is not intended to further limit the claims, but merely to clarify the claimed subject matter.

Because no *prima facie* case of obviousness has been established, Applicant respectfully requests that the rejections under 35 U.S.C. §103 be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing remarks, Applicants respectfully submit that the pending claims are in condition for allowance. An early notice to that effect is earnestly solicited. Should any matters remain outstanding, the Examiner is encouraged to contact the undersigned at the address and telephone number listed below so that they may be resolved without the need for additional action and response thereto.

Respectfully submitted,

Date: February 3, 2003

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Appendix A: Marked-up version of claim amendment

1. (Amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

a) fixing and permeabilizing said cells;

b) catalyzing the deposition of tyramide in said cells comprising said intracellular analyte;

c) [washing] contacting said cells [in] with a medium comprising a chaotropic agent to wash said cells;

d) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby cells comprising said intracellular analyte are specifically labeled; and

e) detecting a signal from cells comprising said detectable label using a flow cytometric device, [whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods] wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

2. (Amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

a) fixing and permeabilizing said cells;

b) catalyzing the deposition of tyramide conjugated to a detectable label in cells comprising said intracellular analyte, whereby cells comprising said intracellular analyte are specifically labeled; and

c) [washing] contacting said cells [in] with a medium comprising a chaotropic agent to wash said cells;

d) detecting a signal from cells comprising said detectable label using a flow cytometric device, [whereby said signal is at least 10-fold greater than a signal obtainable by

standard flow cytometry methods] wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

3. (Amended) [A method according to] The method of claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

4. (Amended) [A method according to] The method of claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

5. (Amended) [A method according to] The method of claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme [capable of catalyzing] that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme;

(ii) removing unbound binding partner from said cells; and

(iii) contacting bound binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

6. (Amended) [A method according to] The method of claim 1 or 2, wherein said detectable label is a fluorochome.

7. (Amended) [A method according to] The method of claim 6, wherein said fluorochome comprises a fluorescent molecule selected from the group consisting of fluoroscein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridenin chlorophyll, and cyanine.

8. (Amended) [A method according to] The method of claim 5, wherein said enzyme is selected from the group consisting of hydrolysases, peroxidases, oxidase, esterases, glycosidases and phosphatases.

9. (Amended) [A method according to] The method of claim 5 wherein said enzyme is horseradish peroxidase.

10. (Amended) [A method according to] The method of claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a first binding parts that specifically binds to said analyte, and a second binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme [capable of catalyzing] that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme the deposition of tyramide;

(ii) removing unbound second binding partner from said cells; and

(iii) contacting bound second binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular anlayte.

11. (Amended) [A method according to] The method of claim 10, wherein said second binding partner is an imunoglobulin-enzyme conjugate.

12. (Amended) [A method according to] The method of claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

13. (Amended) [A method according to] The method of claim 12, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkar cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

14. (Amended) [A method according to] The method of claim 1 or 2 wherein, said one or more cells are cultured cells.

15. (Amended) [A method according to] The method of claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organeller antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.

16. (Amended) [A method according to] The method of claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.

17. (Amended) [A method according to] The method of claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.

18. (Amended) [A method according to] The method of claim 1 or 2, wherein said one or more cells are obtained from a patient.

19. (Amended) [A method according to] The method of claim 18, wherein said signal is correlated to a diagnosis of a disease in said patient.

20. (Amended) A kit for performing a method according to claims 1 or 2, wherein said kit comprises a medium comprising a chaotropic agent; an analyte-specific binding partner conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme; a substrate for said enzyme; and a tyramide reagent selected from the group consisting of unlabeled tyramide and tyramide conjugated to a detectable label, wherein if said tyramide reagent is unlabeled tyramide, said kit further comprises a tyramide-specific binding partner conjugated to a detectable label.

37. (New) The method of claim 1 or 2, wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.